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RESEARCH PAPER

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A study on physiological, anatomical characterization of selected carrot plant under different treatments of salts

Minahal Akram¹, Muhammad Abu Bakar^{*1}, Uzma Nasrullah¹, Shamsa Bano¹,
 Azqa Nawaz¹, Shazia Parveen¹, Muheb Ul Nabi¹, Rana Zeeshan Zulfiqar²,
 Sumera¹, Saadia Bashir¹, Safina¹

¹*Department of Botany, University of Agriculture, Faisalabad, Pakistan*

²*Department of Biochemistry, University of Management and Technology, Lahore, Pakistan*

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Abstract

Carrots were first used for medical purposes and gradually used as food. It is also a good source of magnesium and manganese. Cadmium is a nonessential element that adversely affects plant growth and development. It is considered as one of the significant pollutants due to its high toxicity and more solubility in water. Experiment was conducted in Old Botanical Garden of University of Agriculture Faisalabad to check the response of carrot genotypes grown under Cadmium Chloride stress (0 μ M, 10 μ M, 15 μ M and 20 μ M). Variety of carrot (gajar) used was Red Gold. Seeds were sown in 12 pots, which were filled with sand, in 2nd week of November. Germination observed after one week of sowing. The experiment was laid out in a completely randomized design with three replicates. Seeds of carrot (red gold) were purchased from Ayyub Agriculture Research Institute (AARI) Faisalabad and sown directly in the plastic pot. 8 seeds per pot were distributed. Hogland solution was also applied to replicates and then I applied Cadmium Chloride stress to the plants to start my experiment. Cadmium stress decreased the uptake and distribution of essential elements in plant. Studies had revealed that heavy metals cause adverse effects on plant growth, which further lead to decrease plant yield and inhibition of enzymatic activities. In the present study, plant growth characteristics, root length and shoot length decreased under Cd stress. In fact, salt stress decreased all attributes in carrot when the concentrations of Cadmium chloride increased higher and higher.

***Corresponding Author:** Muhammad Abu Bakar ✉ sayem.ali854@gmail.com

Introduction

Carrot (*Daucus carota* L.) is the most important crop of Apiaceae family. It is a root vegetable that has worldwide distribution. Carrots were first used for medical purposes and gradually used as food. Written records in Europe indicated that carrots were cultivated prior to the tenth century. The colors of the carrot root flesh may be white, yellow, orange, red, purple, or very dark purple. The first cultivated carrots were yellow and purple fleshed cultivars. Orange carrots, today more popular, were developed in the 15th and 16th centuries in Central Europe. A rapid rise in the popularity of orange carrots was observed with the recognition of its high pro vitamin. Vegetables are essential in human diet as they provide plant fiber and vitamins (Abberton *et al.*, 2016).

The yellow/orange color of western carrots is caused by the plastid-bound pigment carotenoids, carotene, and xanthophyll. White carrots contain only traces of pigment, mainly carotene and xanthophyll. The yellow and white types probably originated by mutation. Purple carrots contain anthocyanins, a powerful antioxidant, whilst red contain lycopene, good for eye health, and also found in tomato (Adomas *et al.*, 2008)

Carrot is a good source of dietary fiber and of the trace mineral molybdenum, rarely found in many vegetables. Molybdenum aids in metabolism of fats and carbohydrates and is important for absorption of iron. It is also a good source of magnesium and manganese. Magnesium is needed for bone, protein, making new cells, activating B vitamins, relaxing nerves and muscles, clotting blood, and in energy production. Insulin secretion and function also require magnesium. Manganese is helpful in carbohydrate metabolism, in coordination with enzymes in the body (Baker *et al.*, 2011).

Cadmium is a nonessential element that adversely affects plant growth and development. It is considered as one of the significant pollutants due to its high toxicity and more solubility in water. The monitoring limit of cadmium (Cd) in agricultural soil

is 100mg/kg soil. Concentration of cadmium varies extensively among plant species and varieties within species. Cadmium absorption and translocation varied within different cultivars. The variation in Cd uptake among cultivars may be due to genotypic variations in cultivars to absorb or translocate specific toxic metals. Cadmium can alter the uptake of mineral nutrients by plants through its effects on availability of nutrients from the soil. Plants grown in soil containing high levels of Cd show visible symptoms of injury reflected in terms of chlorosis, leaf rolls, growth inhibition, browning of root tips, and finally death (Bardhan *et al.*, 2013).

Cadmium can alter the uptake of minerals from soil. The level of Cd in soil increases with increase in time. Plants can easily uptake cadmium and transfer it to other organs. In humans, Cd accumulates mainly in the kidney with a biological half-life about 20 years, and leads to pulmonary emphysema and renal tubular damage. Cd has been considered as an extremely significant toxic pollutant affecting all organisms because of its high great solubility in soil and water. Developmental stages of plant such as seedlings and seed germination are more sensitive to various environmental factors like temperature, light intensity, heavy metals pollution (Baker *et al.*, 2011).

The accumulation of Cd in roots and above-ground organs has been investigated in various species, mainly in terms of atomic absorption/emission spectrophotometric analysis, in order to gain quantitative information that might be of interest for basic and applied research on metal tolerance. However, much less effort has been dedicated to investigating the spatial and temporal relationships of Cd effects on: i) cyto-histology, ii) ultrastructure, iii) parallel mounting of structural and functional responses addressed to circumscribe the metal toxicity and, above all, iv) determination of the metal localization sites in planta, by means of Cd-sensitive fluorochromes, use of which is still an unusual technique in plant tissues (Admoas *et al.*, 2008).

The aim of this study was effect of the cadmium against carrot. It also included the microscopic

examination of the carrot to check the growth parameters under different conditions with supplementation of the nutrient medium and different phases of the inoculation were observed during the study and to check the different rates of the bacteria.

Materials and methods

Experiment was conducted in Old Botanical Garden of University of Agriculture Faisalabad to check the response of carrot genotypes grown under Cadmium Chloride stress ($0\mu\text{M}$, $10\mu\text{M}$, $15\mu\text{M}$ and $20\mu\text{M}$). Variety of carrot (gajar) used was Red Gold. Seeds were sown in 12 pots, which were filled with sand, in 2nd week of November. Germination observed after one week of sowing. The experiment was laid out in a completely randomized design with three replicates.

Sowing and culture medium

Seeds of carrot (red gold) were purchased from Ayyub Agriculture Research Institute (AARI) Faisalabad and sown directly in the plastic pot. 8 seeds per pot were distributed. The pots used for sowing contain an underneath hole which was covered by a piece of fine cotton cloth. Each pot was filled with 2.5kg of sand from Old Botanical Garden. After germination, seedlings were thinned to maintain 5 plants per pot of equal size. Hogland solution was also applied to replicates and then I applied Cadmium Chloride stress to the plants to start my experiment.

Treatments and Source

Simple water used from the filler plants present in the University of Agriculture, Faisalabad. The salt that we want to apply to plants are taken from our Botany Department in a specific amount and make the solution in 1L of water and apply to specific plants.

- 1- Normal water or $0\mu\text{M}$ Cadmium Chloride solution,
- 2- $10\mu\text{M}$ Cadmium Chloride solution
- 3- $15\mu\text{M}$ Cadmium Chloride solution and
- 4- $20\mu\text{M}$ Cadmium Chloride solution

Harvests

Plants were harvested after 45 days of treatment and following parameters were studied.

Shoot and Root length

Shoot and root length was measured by using a meter rod and mean values were calculated.

Shoot and Root fresh weight

Fresh weight of shoot and root of plants were determined immediately after uprooting the plants with the help of electrical balance and mean values were calculated.

Shoot and Root dry weight

Shoots were air dried for two days and then placed in oven at 65°C for 7 days to get constant dry weight and mean values were calculated. Shoot dry weight was recorded with the help of electrical balance.

Statistical analysis

The recorded data was computed and analyzed by using analysis of variance technique (ANOVA) by using latest computer software packages COSTAT V 6.3

Results and discussions

Shoot Fresh Weight (g)

Cadmium chloride at $20\mu\text{M}$ has significantly decreased the shoot fresh weight while $0\mu\text{M}$ has maximum shoot fresh weight. That is also controlled medium. Cadmium Chloride stress causes significant ($P \leq 0.01$) effect on shoot fresh weight of carrot .

Shoot Dry Weight (g)

Analysis of variance of data for shoots dry weight of carrot genotype grown under control and Cadmium Chloride condition is presented. Application of Cadmium Chloride caused a nonsignificant reduction in shoot dry weight. Maximum shoot dry weight was observed at control condition ($0\mu\text{M}$ Cadmium chloride); whereas maximum reduction at $20\mu\text{M}$.

Root Fresh Weight (g)

Cadmium chloride at $20\mu\text{M}$ has significantly decrease the root fresh weight while maximum root fresh weight was observed at $0\mu\text{M}$. Cadmium Chloride stress causes nonsignificant effect on root fresh weight of carrot, which is presented.

Root Dry Weight (g)

Analysis of variance of data for roots dry weight of carrot genotype (red gold) grown under control and Cadmium Chloride condition is presented. Application of Cadmium Chloride caused a nonsignificant reduction in root dry weight. Maximum root dry weight was observed at control condition (0 μ M Cadmium chloride); Root dry weight at 10 μ M and 20 μ M remained same whereas maximum reduction occurs at 20 μ M.

Root length (cm)

Cadmium chloride at 20 μ M has significantly decrease the root length of carrot, while 0 μ M has maximum root length. 0 μ M is also controlled medium. Root length of carrot gradually decreased by increasing the CdCl concentration. Cadmium Chloride stress causes significant ($P \leq 0.001$) effect on root length of carrot.

Shoot length (cm)

Analysis of variance of data for the concentration of shoot length in carrot genotype (red gold) grown under controlled and Cadmium Chloride stress condition, is presented. Application of Cadmium Chloride stress caused a significant ($P \leq 0.01$) decrease in shoot length. Maximum increase was observed at 0 μ M, which was controlled medium for the estimation of shoot length, whereas maximum reduction occur at 20 μ M.

Number of branches (per plant)

Analysis of variance of data for number of branches of carrot per plant genotype (red gold) grown under control and Cadmium Chloride condition is presented. Application of Cadmium Chloride caused a nonsignificant reduction in number of branches of carrot per plant. Maximum number of branches of carrot per plant was observed at control condition (0 μ M Cadmium chloride); whereas reduction at 20 μ M.

Table 1. Analysis of Variance of data for shoot fresh weight of Carrot under Cadmium chloride stress.

SOV	Df	SS	MS	F	P
Treatment	3	15.602	5.201	15.183	.0012 **
Error	8	2.740	0.343		

***, ** and * = Significant at 0.001, 0.01 and 0.05 levels respectively. ns= Non significant

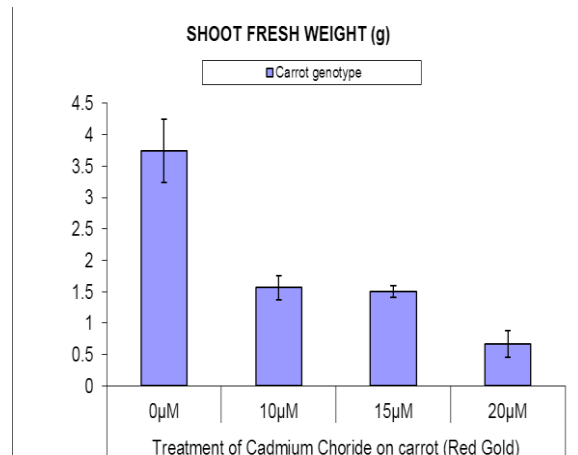


Fig. 1. shoot fresh weight of carrot under cadmium chloride stress.

Table 2. Analysis of Variance of data for shoot dry weight of Carrot (Red Gold) under Cadmium chloride stress.

SOV	df	SS	MS	F	P
Treatment	3	0.114	0.038	20.959	0.0004 ***
Error	8	0.015	0.002		

***, ** and * = Significant at 0.001, 0.01 and 0.05

levels respectively. ns= Non significant

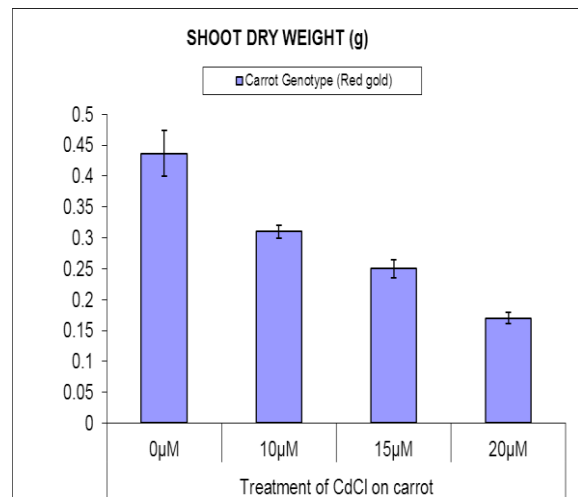


Fig. 2. shoot dry weight of carrot under cadmium chloride stress.

Table 3. Analysis of Variance of data for root fresh weight of Carrot (Red Gold) under Cadmium chloride stress.

SOV	Df	SS	MS	F	P
Treatment	3	0.565	0.188	3.3593634	0.0757 ns
Error	8	0.448	0.056		

***, ** and * = Significant at 0.001, 0.01 and 0.05

levels respectively. ns= Non significant

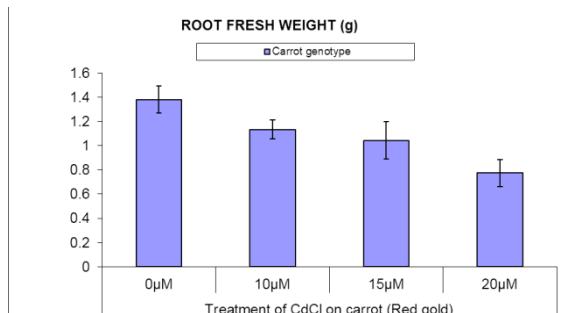


Fig. 3. Root fresh weight of carrot under cadmium chloride stress.

Table 4. Analysis of Variance of data for root dry weight of Carrot (Red Gold) under Cadmium chloride stress.

SOV	Df	SS	MS	F	P
Treatment	3	0.002	6.973	0.1334397	0.9374 ns
Error	8	0.042	0.005		

***, ** and * = Significant at 0.001, 0.01 and 0.05 levels respectively. ns= Non significant

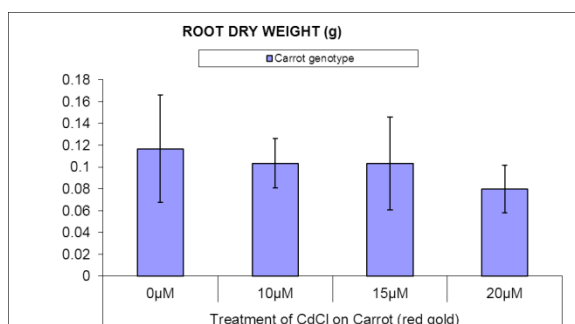


Fig. 4. Root dry weight of carrot under cadmium chloride stress.

Table 5. Analysis of Variance of data for root length of Carrot (Red Gold) under Cadmium chloride stress.

SOV	df	SS	MS	F	P
Treatment	3	33.009	11.003	18.916	0.0005 ***
Error	8	4.653	0.582		

***, ** and * = Significant at 0.001, 0.01 and 0.05 levels respectively. ns= Non significant

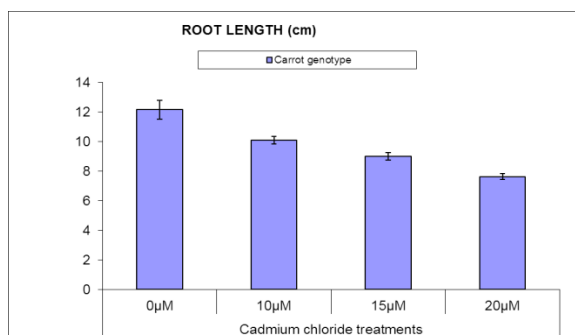


Fig. 5. Root length of carrot under cadmium chloride stress.

Table 6. Analysis of Variance of data for shoot length of Carrot (Red Gold) under Cadmium chloride stress.

SOV	Df	SS	MS	F	P
Treatment	3	575.329	191.776	13.9296	0.0015 **
Error	8	110.14	13.767		

***, ** and * = Significant at 0.001, 0.01 and 0.05 levels respectively. ns= Non significant

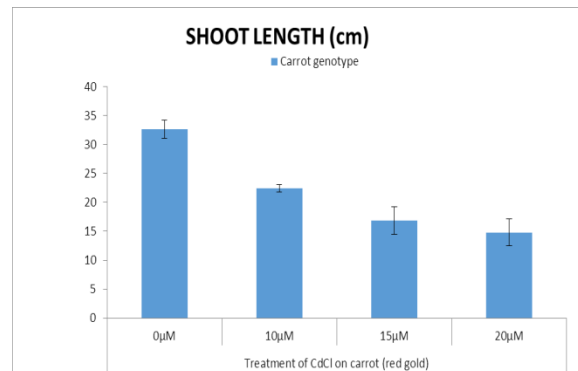


Fig. 6. Shoot length of carrot under cadmium chloride stress.

Table 7. Analysis of Variance of data for No. of branches of Carrot (Red Gold) under Cadmium chloride stress.

SOV	df	SS	MS	F	P
Treatment	3	0.25	0.083	0.1428	0.9314 ns
Error	8	4.667	0.584		

***, ** and * = Significant at 0.001, 0.01 and 0.05 levels respectively. ns= Non significant

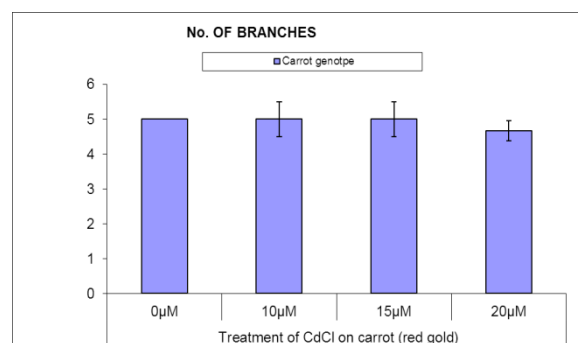


Fig. 7. No. of branches of carrot under cadmium chloride stress.

Cadmium stress decreased the uptake and distribution of essential elements in plant. Studies had revealed that heavy metals cause adverse effects on plant growth, which further lead to decrease plant yield and inhibition of enzymatic activities (Bainbridge *et al.*, 2008)

In the present study, plant growth characteristics, root length and shoot length decreased under Cd stress. Cadmium-induced inhibition in plant growth and biomass has already been reported in many plant species such as wheat. Decrease in plant growth and biomass might be due to Cd-induced toxicity on photosynthetic apparatus and/or structural alterations in plants. Decrease in plant growth and biomass might also be due to oxidative damage and reduction in antioxidant enzymes activities and/or reduction in mineral nutrients uptake by plants. All Cd treatments caused a significant increase in Cd concentrations in roots followed by stem and leaves of cotton plants. Higher Cd concentrations in roots under Cd stress might be a strategy of plants to cope with metal stress as suggested by many researchers (Li *et al.*, 2019).

The leaf fresh weight was more affected by Cd. The dry weight of root, stem and leaf was lower in plants were also decreased. The low fresh weight indicates the adverse impact of heavy metal in plants. Cadmium toxicity is often described as decreased root length and dry mass. Plant accumulates large portion of heavy metal in root followed by stem and leaf. The dry biomass of both roots and shoots was significantly reduced in Cd-treated plants compared to the control plants. Some findings also suggested that plant species have variety of capacities to accumulate specific heavy metals. Roots and leaves of herbaceous plant retain higher concentration of heavy metal than stems and fruits (Meng *et al.*, 2019).

Inhibition of root elongation has been shown to be one of the earliest and distinct symptoms of Cd toxicity. The toxic effect of Cu on root growth recorded more in plants and that of Cd was observed throughout the growth period. Cd metal lowered the stem height of plants. High concentration of Cd caused decrement in leaf number (Cooke *et al.*, 2004). The leaf was the least affected organ. It can be concluded that the organ sensitivity was in the order root>stem>leaf. Increase in accumulation of Cd in plants tends to reduction in formation of new cells which leads to reduction in shoot and root lengths. The vegetative and reproductive growth was reduced

by Cd application. The root elongation was most sensitive process. Cd is well known among all other highly toxic environmental element because of its higher toxicological properties and high mobility from soil to root, root to higher plant parts and further down the food chain. It can be easily accumulated in large amounts in the body of all organisms and alter physiological metabolism processes like photosynthesis, respiration, transpiration and nitrogen assimilation (Van *et al.*, 2012)

Conclusion

Imposition of salt stress in the nutrient medium made a tremendous increase in the accumulation of Carotenoid contents in plant. In all the above attributes Carrot showed best results in control and showed lower result in 20µM concentration. In fact, salt stress decreased all attributes in carrot when the concentrations of Cadmium chloride increased higher and higher.

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